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THE COMPOSITION AND FLUIDITY OF ADIPOCYTE MEMBRANES PREPARED FROM YOUNG AND ADULT RATS

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Summary

Membranes from adipocytes of adult and young rats have been compared. Phospholipid fatty acids from adult rats were more saturated than those from young rats. This difference was associated with a decreased fluidity in the membranes of the adult rats, which was inferred from measurements of fluorescence polarisation of the fluorescent probe, 1,6-diphenylhexa-1,3,5-triene.

Studies with model membrane systems have shown that the fluidity of the membrane matrix is sensitive to the presence of cholesterol and to the degree of saturation of the phospholipid fatty acids [1]. The sensitivity is particularly important since the behaviour of various membrane proteins has been shown to be affected by changes in the fluidity of their environment [2]. Thus, changes in membrane fluidity could have important physiological consequences. Indeed, evidence that eukaryotic organisms attempt to maintain a constant membrane fluidity can be adduced from studies of fluorescent probes in goldfish synaptosomes. These have shown [3] that the composition of phospholipid fatty acid in fish acclimatised to different temperatures is altered in a way which at least partially compensates for the effect of temperature on fluidity; this phenomenon has been termed 'homeoviscous adaptation' [4].

We report here some evidence that when differences in membrane fluidity do arise they can be associated with physiological changes in tissue. We have prepared membranes from adipocytes of young adult rats and have shown that the phospholipid fatty acids from the older, insulin-resistant animals are more saturated than those from the younger animals. This change in composition is accompanied by a decrease in membrane fluidity which has been inferred from

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measurements of the fluorescence polarisation of the fluorescent probe 1,6-diphenylhexa-1,3,5-triene.

Adipocytes were isolated as described previously [5] from the epididymal pads of fed Wistar rats. Young (6-week-old) rats weighed 100—120 g and typically had fat pads which weighed about 0.12 g and yielded about 60 μl of packed adipocytes. Adult (6—9 month) rats weighed 350—450 g and typically had fat pads which weighed about 1.7 g and yielded about 1.5 ml of packed adipocytes.

The washed cells were lysed by osmotic shock [6] using hypotonic 10 mM Tris (pH 7.4) containing 1 mM EDTA. The resulting ghosts (many of which contained nuclei) were harvested by centrifuging at 1000 $\times g$ for 15 min at 4°C; the ghost pellet was resuspended by homogenisation with a Teflon pestle in 3 ml of Tris made isotonic by the addition of 0.25 M sucrose.

Ghosts were layered on a density gradient of 45 and 25% sucrose in hypotonic Tris. Membranes (from which nuclei had been extruded) were collected from the 25/45 interface after centrifuging at 4°C for 40 min at 21 000 rev./min. Membrane lipids were extracted with chloroform : methanol (2 : 1, v/v). Phospholipid phosphate was determined essentially as described by Bartlett [7] and cholesterol by gas-liquid chromatography [8]. Membrane protein was determined by using the method of Lowry et al. [9]. The molar ratio of phospholipid to cholesterol was 1.2 : 1 and the weight ratio of protein to total lipid varied from 1.3 : 1 to 1.4 : 1. The membranes contained 2—5% of the total succinate dehydrogenase [10], NADH : cytochrome *c* reductase [11], DNA [12] and RNA [12] but 30—50% of the 5'-nucleotidase [13].

Standardised solutions of adipocyte triglyceride, triglyceride fatty acid and samples of the lipid fraction containing a known amount of phospholipid were separated by thin-layer chromatography using hexane/diethyl ether/acetic acid (40 : 20 : 1). The amount of triglyceride and fatty acid in the membrane preparation was estimated by developing the plate with iodine vapour and comparing the size and intensity of the relevant spots in the sample and standards. We estimate that neither constituent constitutes as much as 1% of the total phospholipid.

Samples of the dried lipid extract were methylated [14] and the resulting methylated fatty acids were separated by gas-liquid chromatography using a 5 ft glass column packed with 10% Silar 10 CP on 100/120 mesh Gas Chrom Q. The column temperature was 160°C with a nitrogen flow rate of 100 ml/min. The results of five such analyses are shown in Table I. The proportion of saturated fatty acids is consistently higher in the membranes prepared from the adult rats. In particular the adults have more palmitic acid (C 16 : 0) and less oleic acid (C 18 : 1) than the young rats.

1,6-Diphenylhexa-1,3,5-triene was obtained from Sigma Chemical Co. and used as a probe of membrane fluidity [15]. A suspension of membranes containing about 100 μg of membrane protein in phosphate-buffered saline [16] was added to a suspension of the probe to give a final concentration of $0.3 \cdot 10^{-6}$ M diphenylhexatriene. The probe was allowed to equilibrate with the membranes by incubating at 37°C for 30 min in a shaking water bath. The sample was re-equilibrated to 25°C before making fluorescence measurements.

Fluorescence polarisation was measured with a Hitachi-Perkin Elmer MPF-

TABLE I

RATIOS OF THE DIFFERENT FATTY ACIDS OBTAINED BY METHANOLYSIS OF PHOSPHOLIPIDS FROM FIVE DIFFERENT PREPARATIONS OF ADIPOCYTE MEMBRANES

Standard deviations are shown when more than two observations were made on a single preparation. The fatty acids are designated according to the number of carbon atoms in the chain and the number of double bonds.

		Young		Adult		
	Number of observations:	1	4	2	3	4
Fatty acid	16:0	20.3	19.4 ± 1.1	24.0	27.6 ± 0.8	24.0 ± 1.1
	16:1	8.0	5.3 ± 1.3	8.6	6.5 ± 0.2	7.8 ± 0.9
	18:0	16.1	14.1 ± 0.8	13.8	15.4 ± 0.6	15.0 ± 0.8
	18:1	25.7	29.0 ± 1.5	17.8	23.2 ± 0.4	21.7 ± 0.4
	18:2	19.0	22.8 ± 2.0	25.1	17.8 ± 0.5	22.8 ± 0.6
	20:4	10.8	9.3 ± 2.6	10.9	9.5 ± 0.8	9.0 ± 0.9
% saturated		36.4	33.5	37.8	43.0	39.0

2A spectrofluorimeter using excitation and emission wavelengths of 365 and 425 nm, respectively. The fluorescence intensities of membranes in the absence of diphenylhexatriene were negligible at these wavelengths. Measurements were made with illumination periods limited to 10 s in order to minimise the effect of photoisomerisation [17,18]. Fluorescence polarisation was corrected for instrument polarisation as described by Penzer [19].

The fluorescence polarisation of the probe equilibrated with membranes was observed on replicate samples from two preparations of membranes obtained from eight young rats and from two preparations of membranes obtained from three adult rats. The values obtained for young rats were 0.230 ± 0.009 and 0.229 ± 0.009 (7), and for adults 0.243 ± 0.013 (4) and 0.244 ± 0.013 (7) (where the errors are the standard deviations of the number of observations shown in brackets). These data show that there is no reason to suppose that there is any difference between the different preparations made from rats of similar size. It is therefore sensible to combine all the values from the adult rats and all those from the young rats. The Student's *t*-test shows that there is less than 1% probability that the values obtained from membranes of the adults are the same as those obtained from the membranes of the young rats.

The increase in fluorescence polarisation observed in the membranes from the adult rats is consistent with the location of the probe in a less fluid environment. 1,6-Diphenylhexa-1,3,5-triene has the disadvantage that it is thought to partition into neutral lipid in preference to phospholipid [20]. However, we discount the possibility that it is monitoring a difference in triglyceride contamination between our two preparations partly because the level of triglyceride in our preparations appears to be uniformly low and partly because we have found no difference in the triglyceride fatty acid composition between rats of different ages. We therefore conclude that the probe is monitoring a decrease in fluidity in the membrane matrix of the adult rats. Such a decrease is consistent with the observed change in phospholipid fatty acid composition. We are as yet unable to speculate about the relationship between the composi-

tional and fluidity changes and the development of insulin resistance in the adult rats.

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